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<b>(54) Title:</b> SELECTIVE CELL INACTIVATION IN BLOOD  <b>(57) Abstract</b> <p>Treatment with a set of porphyrin compounds using a photodynamic therapy approach is able selectively to lower elevated levels of activated leukocytes in a leukocyte population. By using compounds with absorption maxima of about 670 nm or higher, irradiation wavelengths can be selected to permit treatment of complex tissues, such as whole blood. This is particularly helpful in subjects containing such elevated levels of T-cell subsets, such as HIV-infected subjects.</p>		

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SELECTIVE CELL INACTIVATION IN BLOODTechnical Field

The invention relates to photodynamic methods for selectively depleting blood or bone marrow of cells important in the progress of certain diseases. More specifically, it concerns methods to reduce selectively the population of activated leukocytes in the blood or bone marrow of subjects with immune dysfunctional disorders, such as patients with autoimmune diseases or patients infected with HIV.

Background Art

Unwanted immune responses, such as those characterizing autoimmune disease, allograft rejection, and associated with diseases which affect the immune system directly are characterized by the presence of activated leukocytes, in particular activated T-cells. Various methods to control activated lymphocytes have been described. For example, U.S. Patent 5,011,684 describes the use of monoclonal antibodies directed to IL-2 receptors characteristic of activated T-cells to control allograft rejection and autoimmunity; U.S. Patent 5,152,380 describes similar materials as well as toxin conjugates to control allergic responses. It is generally recognized that a marker for activated T-lymphocytes is the presence of an IL-2 receptor of high affinity.

The immunodisturbances caused by the human immunodeficiency virus (HIV) have been studied, but the nature of the progress of the disease is still uncertain. However, it has been shown that HIV-infected humans have circulating cytotoxic T-lymphocytes that lyse uninfected CD4<sup>+</sup> cells (Zarling, J.M. et al., J Immunol (1990) 144:2992. It is also generally understood that infection with HIV, while depleting the levels of CD4<sup>+</sup> cells, enhances the levels of CD8<sup>+</sup>, DR<sup>+</sup>, and IL-2R<sup>+</sup> leukocytes.

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As set forth above, IL-2R<sup>+</sup> is considered an activation marker and DR is an HLA marker, and these may be present on cells with additional markers such as CD4 and CD8.

One approach to interfering with the progress of this infection and resultant immunosuppression employs strategies to alter the composition of the blood with respect to various components of the immune system. In one attempt to achieve this, U.S. patent 4,960,408 discloses a process of treating a subject having AIDS-related complex systemically with psoralen, and then extracorporeally treating the T-lymphocytes with ultraviolet radiation of a wavelength absorbed by the psoralen compound. The irradiated T-cells are then returned. This treatment apparently enhances the levels of CD3<sup>+</sup> cells, CD4<sup>+</sup> cells, and CD8<sup>+</sup> cells, although not in a uniform fashion.

U.S. patent 5,095,030, issued 10 March 1992, which is incorporated herein in its entirety by reference, discloses and claims various wavelength-specific cytotoxic agents which are members of a class of compounds with long wavelength absorption maxima that can be generically described as "green porphyrins." The compounds disclosed in the patent are porphyrin derivatives which are modified by a Diels Alder reaction effectively to shift the wavelength of absorption to a longer wavelength. This results in some favorable properties as compared to, for example, hematoporphyrin derivative when these compounds are employed in photodynamic therapy generally. As described in this patent, these cytotoxic agents, when administered systemically, "home" to unwanted cells, in particular to tumor cells or pathogenic viruses and subsequent irradiation with light absorbed by these compounds causes them to transition in such a way as to effect cytotoxicity. It is not believed that the compounds themselves are chemically altered in this process.

It has now been found possible selectively to

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diminish the levels of activated leukocyte subsets which are associated with HIV infection or other immune dysfunction using the green porphyrin compounds such as those described above. This depletion can be effected without side effects on the normally functioning B cell, CD4<sup>+</sup> cell, CD8<sup>+</sup> cell or NK cell populations. Either leukocytes after separation from red blood cells can be treated using the method of the invention or whole blood can be subjected to this treatment.

10 Disclosure of the Invention

The invention provides a method selectively to lower the population of activated leukocytes in subjects showing elevated levels of leukocyte activation markers. The invention method involves treating a body fluid, i.e., bone marrow or blood or appropriate fraction thereof, with a specific class of agents useful in photodynamic therapy, followed by irradiating the blood or fraction with at least one wavelength absorbed by the therapeutic. The treatment can be conducted in vivo, entirely extracorporeally, or partially in vivo and partially ex vivo.

Thus, in one aspect, the invention is directed to a method selectively to reduce the activated leukocyte population in the body fluid of a subject in need of such reduction which method comprises:

25       treating said body fluid or an activated leukocyte-containing fraction thereof with an effective amount of a green porphyrin (Gp) compound; and

30       irradiating said treated body fluid with light comprising at least one wavelength absorbed by said Gp compound.

In another aspect, the invention is directed to a method to treat an HIV-infected patient or other immune dysfunctional patient which method comprises treating at least a portion of said patient's body fluid or fraction thereof containing cells to be depleted with an effective

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amount of the Gp, followed by irradiation.

In still other aspects, the invention is directed to leukocyte-activated blood or plasma or other subfraction which has been depleted of activated leukocytes, of HIV-  
5 infected cells, or of free HIV.

#### Brief Description of the Drawings

Figures 1A and 1B show the effect of Gp and irradiation on various subsets of leukocytes having various markers including B-cells, CD4<sup>+</sup> cells, CD8<sup>+</sup>  
10 cells, DR<sup>+</sup> cells and IL-2R<sup>+</sup> cells in blood from two HIV-infected patients.

Figure 2 shows the relative concentrations of the subsets of leukocytes in blood from a normal donor and from two HIV-infected patients.

15 Figures 3A and 3B show the relative concentrations of subsets of leukocytes in blood from a normal donor and an HIV donor, respectively, before and after treatment with BPD and irradiation.

Figure 3C shows the levels of IL-2R<sup>+</sup> cells specifically before and after treatment with BPD and  
20 irradiation in normal and HIV-infected subjects.

Figure 4 shows inactivation of cell associated HIV by treatment with BPD and irradiation.

Figure 5 shows the generic structures of the various green porphyrins useful in the invention.  
25

Figure 6 shows the structures of preferred green porphyrins--specifically BPD-MA, MPD-MD, BPD-DA and BPD-DB.

#### Modes of Carrying Out the Invention

30 The methods of the invention comprise the treatment of a body fluid, such as bone marrow or blood or appropriate fractions thereof containing abnormally elevated levels of activated leukocytes with a green porphyrin compound, followed by irradiation of the body  
35 fluid or fraction with radiation absorbed by the green

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porphyrin. The method of the invention can be conducted in a variety of protocols -- the two essential elements are assuring that the green porphyrins are associated with the cells whose populations are to be diminished and that the green porphyrin associated with these cells is irradiated with the appropriate wavelength. As used herein, "body fluid" refers to body fluids which contain elevated levels of activated leukocytes or other cells, such as HIV-infected cells, to be depleted. Such body fluids are typically bone marrow or blood or fractions thereof.

The green porphyrins employed in the invention are porphyrin based compounds which have been chemically modified so as to exhibit absorption maxima at 670 nm or higher. Through the use of green porphyrins, radiation wavelengths can be used which are only minimally absorbed by background cells and tissue such as red blood cells. This permits the use of whole blood, as opposed to plasma, in the method of the invention. While use of other compounds which are capable of light absorption, such as psoralen, have been used in leukophoreses procedures involving irradiation, the use of plasma in these procedures is mandated by the absorption wavelength required. The convenience of using whole blood is an important advantage offered by the use of the green porphyrins of the invention.

While bone marrow can also be used as the substrate for the method of the invention, the use of the patient's blood is often more convenient. Thus, in one protocol, whole blood is withdrawn from the subject and treated with a suitable concentration of the green porphyrin for a time period sufficient to permit the green porphyrin to be associated with the desired target cells. The whole blood is then irradiated in the extracorporeal container or apparatus with the appropriate wavelength and the treated irradiated blood returned to the patient.

Alternatively, the patient is administered the green

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porphyrin or mixtures thereof in suitable formulations, typically by injection but also by other routes of administration such as oral administration or transmucosal administration. Liposomal compositions are particularly useful. After the green porphyrin has been given sufficient time to enter the bloodstream, a suitable radiation source can be applied to the body of the patient; for example, a catheterized fiber optic may be inserted into suitable positions in the bloodstream, or external transcutaneous light exposure may be employed. The blood is then irradiated in vivo.

Alternatively, the blood withdrawn from the patient can be separated into fractions, typically into a red blood cell fraction and a leukocyte-enriched plasma fraction and the leukocyte-enriched plasma treated with green porphyrin and irradiated before returning the plasma (and the red blood cells if desired) into the patient. Of course, the separation into fractions can be conducted at any point during the extracorporeal treatment so that the Gp could be added to the whole blood and the blood subsequently separated into red blood cells and leukocyte-enriched fractions before irradiation of the leukocyte-enriched fraction.

Suitable subjects for the treatment of the invention include HIV infected patients who have been shown to have elevated levels of activated leukocytes, such as DR<sup>+</sup>, but especially IL-2R<sup>+</sup> cells in their blood. These levels can be diminished selectively by the method of the invention. Other patients who are expected to show elevated levels of these cells include patients showing unwanted immunoactivation generally, such as those who are subject to graft-versus-host disease or organ transplant rejection as a result, for example, of organ or bone marrow transplantation, and those who show autoimmune conditions such as rheumatoid arthritis, lupus erythematosus, muscular dystrophy, or myasthenia gravis. For transplantation, fluids from the donor organ or

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fluids from the patient recipient may be treated.

Typically, the green porphyrins are administered in such a way as to result in a final concentration in the fluid or fluid fraction to be treated of about 0.05-5  $\mu\text{g/ml}$ , preferably about 0.1-1  $\mu\text{g/ml}$  and most preferably about 0.5  $\mu\text{g/ml}$ . The treated fluid or fluid fraction is then irradiated with light from any suitable source such as a laser diode, a light-emitting diode, fiber-optic-conducted laser light, and the like. Typical wavelengths are in the range of about 600-790 nm, preferably 630-710 nm, most preferably 690-780 nm and typical intensities are on the order of 1-50  $\text{J/cm}^2$ , preferably about 5-25  $\text{J/cm}^2$  and most preferably around 8-15  $\text{J/cm}^2$ . A wavelength range of 685-695 nm for these intensities is preferred. Irradiation is continued for about 2-180 minutes, preferably about 15-120 minutes depending on the nature and concentration of the green porphyrin, the amount of fluid treated, the susceptibility of the patient's cells, the intensity and wavelength of the light, and the method of irradiation (in vivo or ex vivo). Suitable optimization of the concentration of Gp and irradiation parameters is well within ordinary skill.

In further detail with respect to the green porphyrins useful in the invention, the general structures of typical green porphyrins are shown in Figure 5. Particularly preferred forms are shown in Figure 6.

This set of Gp is selected from a group of porphyrin derivatives obtained using Diels-Alder reactions of acetylene derivatives with protoporphyrin under conditions which effect a reaction at only one of the two available conjugated, nonaromatic diene structures present in the protoporphyrin-IX ring system (rings A and B). The formulas shown in Figure 5 represent typical green porphyrins of the invention. These compounds are shown in the figure with hydrogen occupying the internal ring nitrogens; however, it is understood that the

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metalated forms wherein a cation replaces one or both of these hydrogens can also be employed. It is also understood that these compounds can be labeled either by replacement of one or more of the atoms in the structure by its radioactive form, or by coupling to a radioisotope such as a radioactive metal or, for example, a radioisotope of iodine.

For convenience, an abbreviation of the term hydro-monobenzoporphyrin derivative--"BPD"--is generally used to refer to compounds of formulas 3 and 4 of Figure 5. These are the preferred forms of Gp. As shown in Figure 5, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are non-interfering substituents which do not affect, appreciably, the absorption maximum exhibited by the compound. Most typically, R<sup>1</sup> and R<sup>2</sup> are carbalkoxy groups, typically methyl or ethyl carboxy esters. Most commonly R<sup>3</sup> is 2-carboxyethyl or the alkyl ester thereof and R<sup>4</sup> is vinyl. These preferred embodiments result from the availability of native porphyrins and are non mandated by considerations of biological efficacy. By "non-interfering substituents" is meant substituents which neither lower the maximum absorption wavelength below 670 nm nor destroy the ability of the green porphyrin to home to activated leukocytes, nor interfere with the effect of activation of the compound by light on biological tissue.

Dimeric forms of the Gp can also be provided, thus amplifying the ability of the Gp compound to absorb light on a per mole basis. Dimeric and multimeric forms of Gp/porphyrin combinations can also be employed, providing additional absorption wavelengths.

The green porphyrins can be conjugated to specific ligands reactive with a target, such as receptor-specific ligands or immunoglobulins or immunospecific portions of immunoglobulins, permitting them to be more concentrated in a desired target tissue or substances. This conjugation permits further lowering of the required dose levels since the material is not wasted in distribution

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into other tissues whose destruction, far from being desired, must be avoided.

When the Gp is irradiated *in situ* using light in the visible absorption range, photoactivation results in cytotoxicity to the surrounding tissue. While the absorption spectrum also includes shorter wavelengths, there is an especially useful absorption maximum in the 670-780 nm range.

As used herein, "green porphyrin" refers to a porphyrin nucleus modified so as to achieve a wavelength absorption maximum of 670 nm or higher. The absorption maximum is controlled by the conjugated  $\pi$  system of the porphyrin nucleus. In protoporphyrin-IX two of the pyrroles contain vinyl substitutions such that the exocyclic  $\pi$ -bond is conjugated to one of the two  $\pi$ -bonds in the ring. A Diels-Alder reaction involving one of these conjugated systems with an acetylene derivative dienophile results in a fused cyclohexadiene--referred to herein as "hydrobenzo"--fused to the A or B ring, as shown in formulas 1 and 2. Rearrangement of the  $\pi$  system in the hexadiene ring results in the compounds of formulas 3 and 4; reduction provides the compounds of formulas 5 and 6. All of these compounds provide the desired bathochromic shift in absorption maximum.

Specific preparation of a class of Gp compounds useful in the invention is described in detail in the above-referenced U.S. patent 5,095,030.

For the compounds shown in Figures 5 and 6, generally,  $R^1$  and  $R^2$  are each, independently, moderate electron- withdrawing substituents, and are, most commonly, carbalkoxy, or alkyl or aryl sulfonyl, or any other activating substituents, which are not sufficiently electron-withdrawing to result in reaction with both A and B rings rather than reaction with only one, such as cyano or  $-\text{CONR}^3\text{CO}-$  wherein  $R^3$  is aryl or alkyl. One of

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R<sup>1</sup> and R<sup>2</sup> may optionally be H while the other is an electron withdrawing substituent of sufficient strength to facilitate the Diels-Alder reaction.

As used herein, carboxy is, as conventionally defined, -COOH and carbalkoxy is -COOR, wherein R is alkyl; carboxyalkyl refers to the substituent -R'-COOH wherein R' is alkylene; carbalkoxyalkyl refers to -R'-COOR wherein R' and R are alkylene and alkyl respectively. Alkyl is a saturated straight or branched chain hydrocarbyl of 1-6 carbon atoms such as methyl, n-hexyl, 2-methylpentyl, t-butyl, n-propyl, and so forth. Alkylene is as alkyl except that the group is divalent. Aryl or alkyl sulfonyl moieties have the formula SO<sub>2</sub>R wherein R is alkyl as above-defined, or is aryl, wherein aryl is phenyl optionally substituted with 1-3 substituents independently selected from halo (fluoro, chloro, bromo or iodo), lower alkyl (1-4C) or lower alkoxy (1-4C). In addition, one or both R<sup>1</sup> or R<sup>2</sup> can itself be aryl--i.e., phenyl optionally substituted as above defined.

As shown in Figure 5, the adduct formed by the reaction of R<sup>1</sup>-C=C-R<sup>2</sup> with the protoporphyrin-IX ring system (R<sup>3</sup> is a protected form of 2-carboxyethyl such as 2-carbomethoxyethyl or 2-carboethoxyethyl; R<sup>4</sup> is CH=CH<sub>2</sub>) are compounds of the formulas 1 and 2 wherein the compound in formula 1 results from addition to the A ring and formula 2 results from addition to the B ring. In these resulting products of formulas 1 and 2, R<sup>4</sup> remains CH=CH<sub>2</sub>, however this vinyl group is readily derivatized to other embodiments of R<sup>4</sup> by addition to or oxidation of the vinyl ring substituent of ring B in formula 1 or ring A in formula 2. The addition or oxidation products can be further substituted if the added substituents are functional leaving groups--for example -Br may be substituted by -OH, -OR (R is alkyl 1-6C as above), or -NH<sub>2</sub>, -NHR, -NR<sub>2</sub>, etc. In preferred embodiments, one of the added substituents is hydrogen, and the other is

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selected from the group consisting of halo (fluoro, chloro, bromo or iodo), hydroxy, lower alkoxy, amino or an amide, sulfhydryl or an organo-sulfide or can be, itself, hydrogen. Addition to the vinyl group does not appreciably change the absorption spectrum of the resulting compound. The product of the Markonikov addition of water provides a substituent structure analogous to the hematoporphyrin ring system at the relevant ring. Thus, the compounds of the invention include various groups as  $R^4$ , including substituents which provide additional porphyrin or porphyrin-related ring systems, as will be further described below.

$R^3$  in protoporphyrin-IX is 2-carboxyethyl ( $-\text{CH}_2\text{CH}_2\text{COOH}$ ). However, the nature of  $R^3$  (unless it contains a  $\pi$ -bond conjugated to ring  $\pi$ -bond), is ordinarily not relevant to the progress of the Diels-Alder reaction or to the effectiveness and absorption spectrum of the resulting product.  $R^3$  can thus be, for example, lower alkyl (1-4C), or 1-carboxyalkyl (2-6C) or the esters or amides thereof. The  $R^3$  substituent may also be substituted with halogen as above-defined, or with other nonreactive substituents. However, as the convenient starting materials for the Gp compounds of the invention are the naturally occurring porphyrins, the preferred substituents for  $R^3$  are  $\text{CH}_2\text{CH}_2\text{COOH}$  or  $-\text{CH}_2\text{CHR}_2\text{COOR}$ , wherein R is alkyl (1-6C).

In the BPD compounds of the invention, it has been found advantageous to hydrolyze or partially hydrolyze the esterified carboxy group in  $-\text{CH}_2\text{CH}_2\text{COOR}$ . The hydrolysis occurs at a much faster rate than that of the ester groups of  $R^1$ ,  $R^2$ , and the solubility and biodistribution characteristics of the resulting compounds are more desirable than those of the unhydrolyzed form. Hydrolysis results in the diacid or monoacid products (or their salts).

The hydro-monobenzoporphyrins which directly result from the Diels-Alder reaction described in the cited

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references can also be isomerized to compounds of formulas shown as 3 and 4 of Figure 5.

5 The depictions of compounds 3 and 4 in Figure 5 do not show the relative position of the exocyclic methyl group (ring A of formula 3 and ring B of formula 4) with respect to the  $R^2$  substituent. Either isomer is available.

10 In addition, the Diels-Alder products can be selectively reduced by treating with hydrogen in the presence of palladium on charcoal to give the saturated ring analogs, shown as formulas 5 and 6 in Figure 5, corresponding to the respective Diels-Alder products of rings A and B. These reduced products are less preferred  
15 embodiments, and are less useful in the method of the invention than the compounds of formulas 1-4.

The description set forth above with respect to the compounds of formulas 1 and 2 concerning derivatization by conversion of the remaining vinyl substituent ( $R^4$ ) and with respect to variability of  $-R^3$  applies as well to the  
20 compounds of formulas 3, 4, 5 and 6.

The compounds of formulas 3 and 4 (BPD), and especially those which have hydrolyzed and partially hydrolyzed carbalkoxy groups in  $R^3$ , are most preferred. Compounds of the invention which contain  $-COOH$  may be  
25 prepared as the free acid or in the form of salts with organic or inorganic bases.

It will be noted that many of the compounds of Figure 5 contain at least one chiral center and therefore exist as optical isomers. The conjugates and methods of  
30 the invention include compounds having both configurations of the chiral carbons, whether the compounds are supplied as isolates of a single stereoisomer or are mixtures of enantiomers and/or diastereomers. Separation of mixtures of diastereomers may be effected by any  
35 conventional means; mixtures of enantiomers may be separated by usual techniques of reacting them with optically active preparations and separating the

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resulting diastereomers.

It should further be noted that the reaction products may be unseparated mixtures of A and B ring additions, e.g., mixtures of formulas 1 and 2 or 3 and 4 or 5 and 6. Either the separated forms--i.e., formula 3 alone or 4 alone, or mixtures in any ratio may be employed in the methods of therapy and diagnosis set forth herein.

Figure 6 shows four particularly preferred compounds of the invention which have not been previously described in the art. These compounds are collectively designated benzoporphyrin derivative (BPD) as they are forms of Gp having the formula 3 or 4. These are hydrolyzed or partially hydrolyzed forms of the rearranged products of formula 3 and 4, wherein one or both of the protected carboxyl groups of R<sup>3</sup> are hydrolyzed. The ester groups at R<sup>1</sup> and R<sup>2</sup> hydrolyze relatively so slowly that conversion to the forms shown in Figure 6 is easily effected.

For purposes of this description, R<sup>3</sup> is -CH<sub>2</sub>CH<sub>2</sub>COOR<sup>3</sup>. As shown in Figure 6, each R<sup>3</sup> is H in preferred compound BPD-DA, R<sup>1</sup> and R<sup>2</sup> are carbalkoxy, and derivatization is at ring A; BPD-DB is the corresponding compound wherein derivatization is at ring B. BPD-MA represents the partially hydrolyzed form of BPD-DA, and BPD-MB, the partially hydrolyzed form of BPD-DB. Thus, in these latter compounds, R<sup>1</sup> and R<sup>2</sup> are carbalkoxy, one R<sup>3</sup> is H and the other R<sup>3</sup> is alkyl (1-6C). The compounds of formulas BPD-MA and BPD-MB may be homogeneous wherein only the C ring carbalkoxyethyl or only the D ring carbalkoxyethyl is hydrolyzed, or may be mixtures of the C and D ring substituent hydrolyzates. In addition, mixtures of any two or more of BPD-MA, -MB, -DA and -DB may be employed in the method of the invention.

Certain other embodiments wherein R<sup>4</sup> is other than vinyl or wherein R<sup>3</sup> is a nonnative substituent are also included in the Gp useful in the invention.

Generally, each R<sup>1</sup> and R<sup>2</sup> is independently selected

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from the group consisting of carbalkoxy (2-6C), alkyl (1-6C) sulfonyl, aryl (6-10C) sulfonyl, aryl (6-10C); cyano; and  $-\text{CONR}^3\text{CO}-$  wherein  $\text{R}^3$  is aryl (6-10C) or alkyl (1-6C);

each  $\text{R}^3$  is independently carboxyalkyl (2-6C) or a salt, amide, ester or acylhydrazone thereof, or is alkyl (1-6C); and

$\text{R}^4$  is  $\text{CHCH}_3$ ,  $\text{CHOR}^{4'}$ ,  $-\text{CHO}$ ,  $-\text{COOR}^{4'}$ ,  $\text{CH(OR}^{4'})\text{CH}_3$ ,  $\text{CH(OR}^{4'})\text{CH}_2\text{OR}^{4'}$ ,  $-\text{CH(SR}^{4'})\text{CH}_3$ ,  $-\text{CH(NR}^{4'})\text{CH}_3$ ,  $-\text{CH(CN)CH}_3$ ,  $-\text{CH(COOR}^{4'})\text{CH}_3$ ,  $-\text{CH((OOCR}^{4'})\text{CH}_3$ ,  $-\text{CH(halo)CH}_3$ , or  $-\text{CH(halo)CH}_2\text{(halo)}$ ,

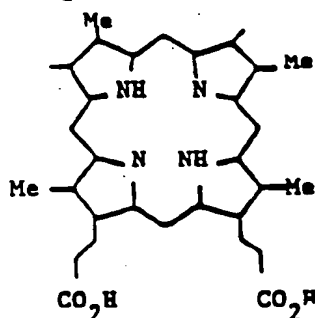
wherein  $\text{R}^{4'}$  is H, alkyl (1-6C) optionally substituted with a hydrophilic substituent, or

wherein  $\text{R}^4$  is an organic group of  $<12\text{C}$  resulting from direct or indirect derivatization of vinyl, or

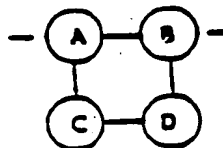
wherein  $\text{R}^4$  is a group containing 1-3 tetrapyrrole-type nuclei of the formula  $-\text{L-P}$  as herein defined.

Compounds of the formulas 3 and 4 and mixtures thereof are particularly preferred. Also preferred are those wherein  $\text{R}^1$  and  $\text{R}^2$  are the same and are carbalkoxy, especially carboethoxy; also preferred are those wherein  $\text{R}^4$  is  $-\text{CHCH}_3$ ,  $\text{CH(OH)CH}_3$ , or  $-\text{CH(halo)CH}_3$ , or is a group containing 1-3 tetrapyrrole-type nuclei of the formula  $-\text{L-P}$  (defined below).

As used herein, "tetrapyrrole-type nucleus" represents a four-ring system of the skeleton:



which is abbreviated



and a salt, ester, amide or acylhydrazone thereof, which is highly conjugated. It includes the porphyrin system, which is, in effect, a completely conjugated system, the chlorin system, which is, in effect, a dihydro form of

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the porphyrin, and the reduced chlorin system, which is a tetrahydro form of the completely conjugated system. When "porphyrin" is specified, the completely conjugated system is indicated; Gp is effectively a dihydro form of the porphyrin system.

One group of compounds is that wherein the substituent  $R^4$  includes at least one additional tetrapyrrole-type nucleus. The resulting compounds of the invention are dimers or oligomers in which at least one of the tetrapyrrole-type ring systems is Gp. Linkage between the Gp moiety through the position of  $R^4$  to an additional tetrapyrrole-type ring system may be through an ether, amine or vinyl linkage. Additional derivatization in the case of porphyrin ring systems which have two available substituent positions (in both A and B rings) corresponding to  $R^4$  can also be formed, as further described below.

As stated above, the compounds of formulas shown in Figure 5 include those wherein the embodiment of  $R^4$  is formed by addition to the vinyl groups of initial Gp products. Thus,  $R^4$  can be any substituent consistent with that formed by a facile addition reaction. Thus, both added substituents can be, for example, OH or halo, and these substituents can be further substituted, or the addition reagent may be of the form HX wherein H is added to the ring-adjacent carbon to provide  $R^4$  of the form

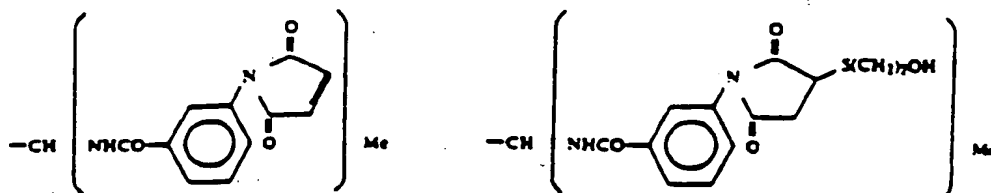
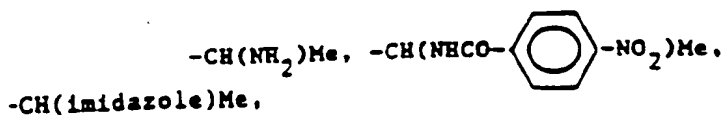
$$\begin{array}{c} -CH_2CH_3 \\ | \\ X. \end{array}$$

The vinyl group can also be oxidized to obtain  $R^4$  as  $CH_2OH$ ,  $-CHO$ , or  $COOH$  and its salts and esters.

Thus, in general  $R^4$  represents any substituents to which the vinyl group  $-CH=CH_2$  is readily converted by cleavage or addition, and further resultants of reaction of leaving groups with additional moieties. Typical  $R^4$  substituents include:

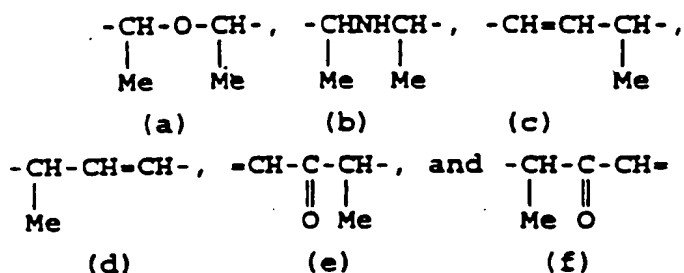
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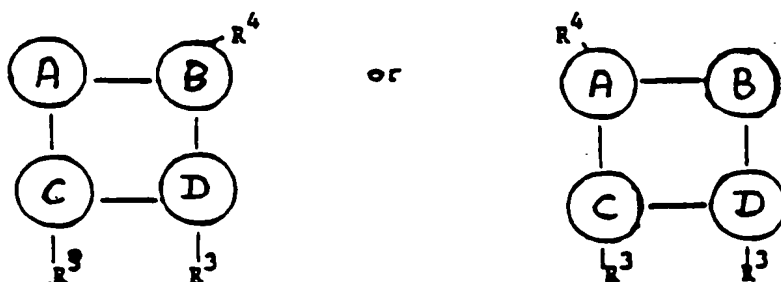


$\text{CH}(\text{OH})\text{Me}, -\text{CHBrMe}, -\text{CH}(\text{OMe})\text{Me}, -\text{CH}(\text{pyridinium bromide})\text{Me},$   
 $-\text{CH}(\text{SH})\text{Me}$  and the disulfide thereof,  $-\text{CHOHCH}_2\text{OH}, -\text{CHO},$  and  
 $-\text{COOH}$  or  $-\text{COOMe}.$

When  $\text{R}^4$  is  $-\text{L-P}$ , the substituent formula " $-\text{L-P}$ " represents a substituent wherein  $-\text{L}-$  is selected the group consisting of



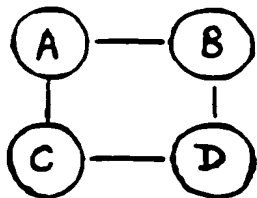
and  $\text{P}$  is selected from the group consisting of  $\text{Gp}$  wherein  $\text{Gp}$  is of the formula 1-6 shown in Figure 5, but lacking  $\text{R}^4$  and conjugated through the position shown in Figure 5 as occupied by  $\text{R}^4$  to  $\text{L}$ , and a porphyrin of the formula



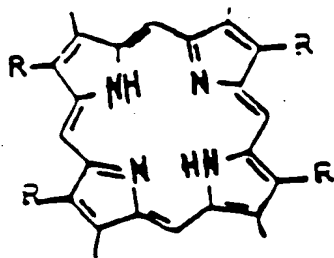
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wherein R<sup>3</sup> and R<sup>4</sup> are as above-defined, and the unoccupied bond is then conjugated to L. It is understood that the abbreviation



represents a porphyrin of the formula:

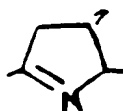


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wherein each R is independently H or lower alkyl (1-4C).

(It is also understood that when -L- is of the formula (e) or (f), the ring system to which the double bond is attached will have a resonance system corresponding to



in the ring to which the double bond is attached, as shown.)

#### Preparation of the Dimers and Oligomers

The dimers and oligomeric compounds of the invention can be prepared using reactions analogous to those for dimerization and oligomerization of porphyrins per se. The green porphyrins or green porphyrin/ porphyrin linkages can be made directly, or porphyrins may be coupled, followed by a Diels-Alder reaction of either or both terminal porphyrins to convert to the corresponding green porphyrin.

In general, the green porphyrins are prepared by any set of reactions which alters the  $\pi$ -bond conjugation system of the porphyrin nucleus so as to shift the wavelength maximum to the required level. Methods for chemically modifying porphyrin to achieve this shift in wavelength maximum are generally known in the art.

The following examples are intended to illustrate

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but not to limit the invention.

Example 1

Response of Various Leukocytes to BPD and Irradiation

5 Blood was withdrawn from two patients who were shown to be infected with HIV. Whole blood from each patient was brought to varying concentrations with BPD-MA and then irradiated with light at  $10.8 \text{ J/cm}^2$  using 690 nm light emitted from LEDs over 4 minutes. The populations of various subsets of T-cells were evaluated by flow  
10 cytometry in comparison to untreated controls and the percentage of cells present as a percent of control plotted as a function of BPD-MA concentration.

The results are shown in Figures 1A and 1B. As indicated, most cell populations remained relatively  
15 constant, including B-cells and  $\text{CD4}^+$  cells. Slight decreases were shown in  $\text{CD8}^+$  cells and  $\text{DR}^+$  cells.  $\text{IL-2R}^+$  cells show a dramatic decrease in the patient results shown in Figure 1A which is dose-dependent on BPD-MA.

20 As shown in Figure 1A, although  $\text{IL-2R}^+$  cells are dramatically decreased, significant decreases for  $\text{DR}^+$  and  $\text{CD8}^+$  cells are also shown. The effect on  $\text{DR}^+$  cells is more dramatically shown for the second patient in Figure 1B.

25 Similar studies were conducted with a constant concentration of BPD-MA ( $0.5 \text{ } \mu\text{g/ml}$ ) using as subjects a normal subject and two HIV patients. Figure 2 shows the subpopulations of leukocytes in untreated blood from these three donors. As shown in Figure 2, B-cells and natural killer cells occur at roughly the same levels in  
30 normals and HIV patients; however, ARC patients consistently show diminished levels of  $\text{CD4}^+$  and elevated levels of  $\text{CD8}^+$ ,  $\text{DR}^+$  and  $\text{IL-2R}^+$ .

35 Figures 3A, 3B, and 3C show the effects of the treatment of the invention. As shown in Figure 3A, treatment of leukocyte subpopulations in normal blood has little effect on any of the populations shown. Figure 3B

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shows that when HIV-infected blood is subjected to this treatment, there is a slight diminution in CD8<sup>+</sup>, a slight diminution in DR<sup>+</sup>, and a significant decrease in IL-2R<sup>+</sup> cells. Figure 3C shows these results specifically for IL-2R<sup>+</sup> cells both for normals and for two HIV-infected patients. Again, the dramatic decrease is shown.

Thus, after treatment with 0.5 µg/ml BPD for 4 minutes in the presence of 10.8 J/cm<sup>2</sup> of light centered at 690 nanometer, the cell populations of all of the cell types in the normal subject, and all cell types except for IL-2R<sup>+</sup> in the HIV patients remains relatively constant. DR<sup>+</sup> and CD8<sup>+</sup> cells are slightly decreased. The BPD/light treatment seems to have little or no effect on most subclasses of leukocytes; however, the elevated levels of IL-2R<sup>+</sup> cells are restored to normal.

#### Example 2

Whole human blood from an HIV patient was also subjected to the treatment using various concentrations of BPD and various light intensities in a protocol similar to that set forth in Example 1 and the effect on inactivation of cell-associated HIV was tested. The results are shown in Figure 4. As shown, intensities of 13 J/cm<sup>2</sup> gave dramatic inactivation of the virus at concentrations of BPD at about 0.5 µg/ml or less. Lower intensities of radiation required higher concentrations of BPD completely to inactivate the virus.

#### Example 3

Inactivation of free HIV (LAV-1 strain) in tissue culture of CEM cells was also tested. In the assay, LAV-1 stock was diluted into the tissue culture medium and BPD was added at either 0.25 µg/ml or 0.5 µg/ml. The media were incubated for 1 hour and exposed to 3 minutes of irradiation centered at 690 nm at an intensity of 10.8 J/cm<sup>2</sup>. The media were then added to CEM cells and the cells were assayed after 6 days of culture using a

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standard p24 assay. The results are read in terms of pg/ml.

The results are shown in Table 1. As shown, at dilutions of LAV that provided high levels of p24, treatment with BPD at either 0.25  $\mu\text{g/ml}$  or 0.5  $\mu\text{g/ml}$  was able substantially to lower the level of p24 determined.

Table 1  
 $\bar{x}$  pg/ml (p24)

	LAV-1	TREATMENT	TREATMENT	TREATMENT
	DILUTION	No Drug no light	.25 $\mu\text{g}$ BPD 3 min	.5 $\mu\text{g}$ BPD 3 min
10	$10^{-1}$	>616	23.2	14.2
	$3 \times 10^{-1}$	>616	18.2	14.5
	$5 \times 10^{-1}$	>616	15.1	11.4
15	$7 \times 10^{-1}$	>616	8.9	7.6
	$9 \times 10^{-1}$	>616	7.6	5.4
	$10^{-3}$	>616	4.5	7.0
	$3 \times 10^{-3}$	>616	5.1	3.2
	$5 \times 10^{-3}$	>616	4.5	3.9
20	$7 \times 10^{-3}$	>616	3.9	2.0
	$9 \times 10^{-3}$	>616	5.4	2.3
	$10^{-4}$	>616	2.0	4.2
	$3 \times 10^{-4}$	>616	3.6	2.0
	$5 \times 10^{-4}$	>616	2.9	2.3
25	$7 \times 10^{-4}$	>616	3.2	-
	$9 \times 10^{-4}$	2.6	2.6	-

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Claims

1. A method selectively to reduce the  
activated leukocyte cell population in an activated  
leukocyte-containing fluid which method comprises:  
5       treating said fluid or an activated  
leukocyte-containing fraction thereof with an effective  
amount of a green porphyrin (Gp) compound, and  
      irradiating said treated fluid or fraction  
with light comprising at least one wavelength absorbed by  
10       said Gp compound.

2. The method of claim 1 wherein said  
treating results in a Gp concentration of about 0.05-5  
µg/ml.

3. The method of claim 1 wherein the fluid  
15       is whole blood.

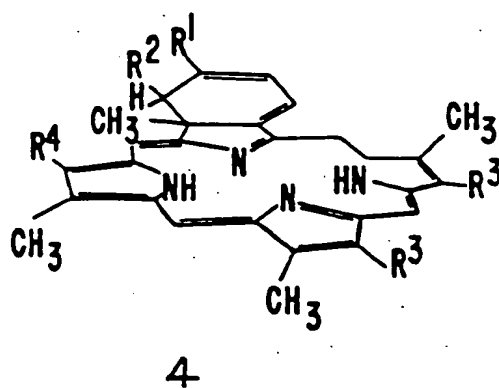
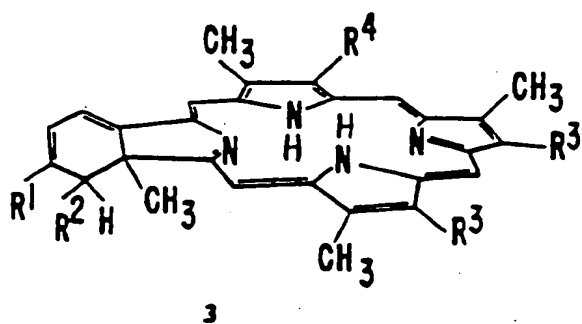
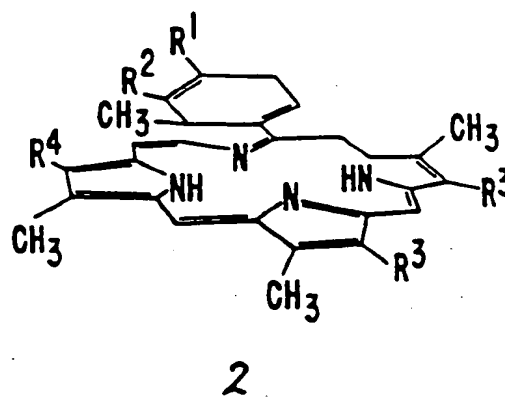
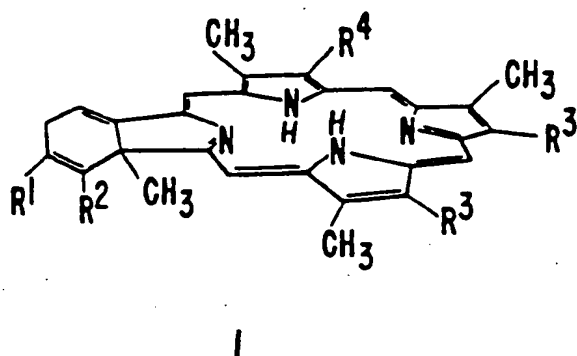
4. The method of claim 1 wherein the fluid  
is contained in an animal subject.

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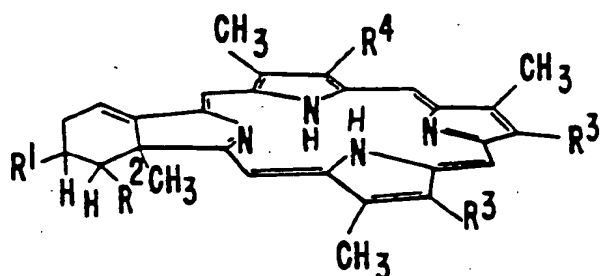
- 23 -

5. The method of claim 1 wherein said Gp has a formula selected from the group consisting of

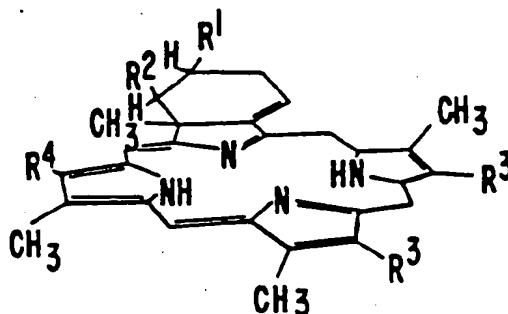


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and



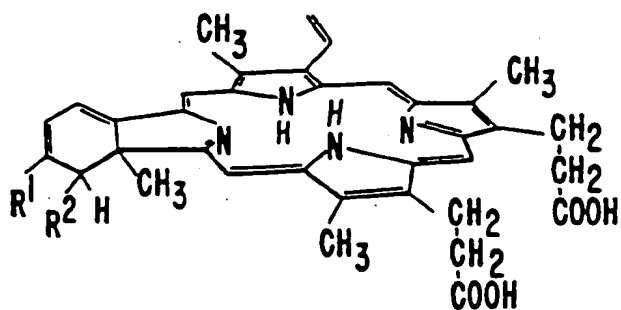
wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are non-interfering substituents.

6. The method of claim 5 wherein said Op is of formula 3 or 4, R<sup>1</sup> and R<sup>2</sup> are carbomethoxy or carboethoxy, each R<sup>3</sup> is -CH<sub>2</sub>CH<sub>2</sub>COOH- or a salt, amide, ester or acyl hydrazone thereof; and R<sup>4</sup> is vinyl.

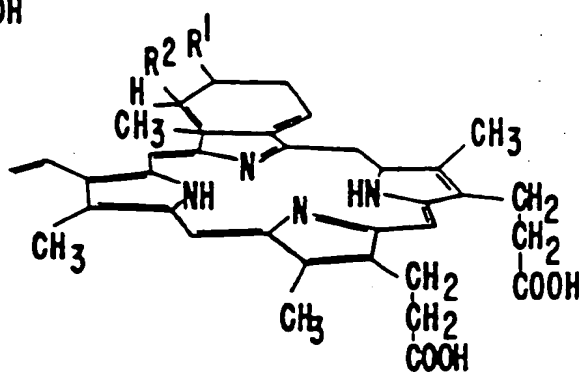
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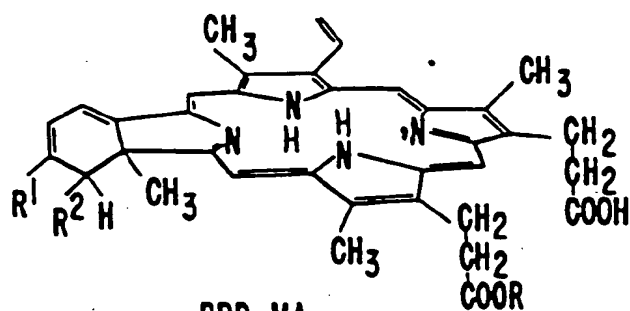
7. The method of claim 6 wherein said Gp has a formula selected from the group consisting of



BPD-DA

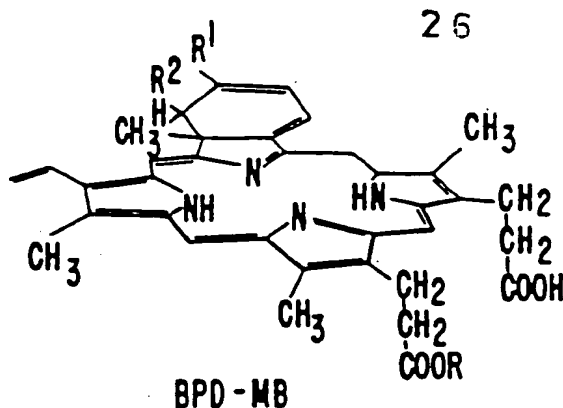


BPD-DB



BPD-MA

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wherein each of  $R^1$  and  $R^2$  is independently selected from carbomethoxy and carboethoxy, and R is methyl or ethyl.

8. Body fluid or a fraction thereof which has been depleted of activated leukocytes by the method of claim 1.

9. Blood or a fraction thereof which contains elevated levels of  $CD8^+$  cells and of  $DR^+$  cells, but normal levels of  $IL-2R^+$  cells.

10. A method to treat the body fluids of a patient with immune dysfunction which method comprises treating at least a portion of said patient's leukocyte-containing body fluid or a fraction thereof containing activated leukocytes with an effective amount of a green porphyrin (Gp) compound, and

irradiating said treated fluid with light comprising at least one wavelength absorbed by said Gp compound.

11. The method of claim 10 wherein said patient is HIV-infected or wherein said patient has an autoimmune disease, or wherein said patient is a transplant recipient.

12. The method of claim 10 wherein said

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fluid is whole blood.

13. A method to inactivate HIV virus which method comprises contacting a fluid containing said virus with an effective amount of a green porphyrin (Gp) compound, and

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irradiating said treated fluid with light comprising at least one wavelength absorbed by said Gp compound.

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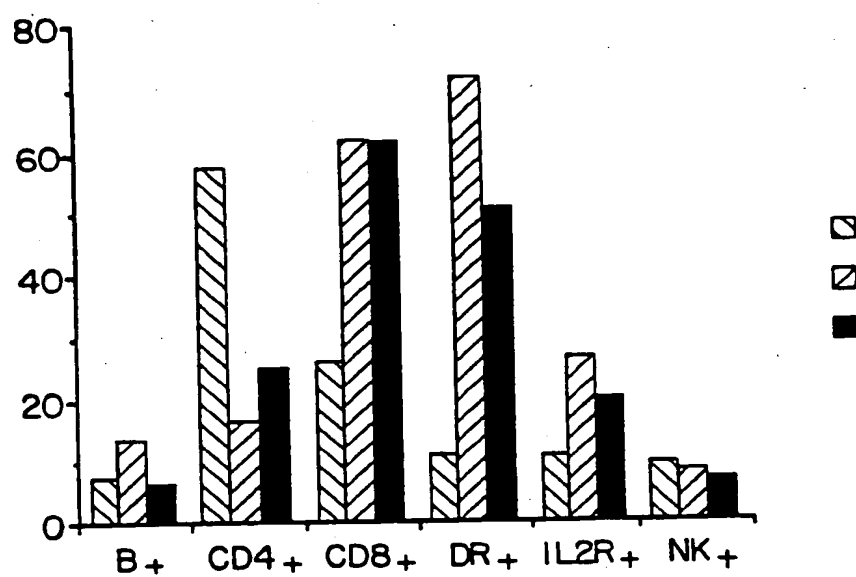


FIG. 2

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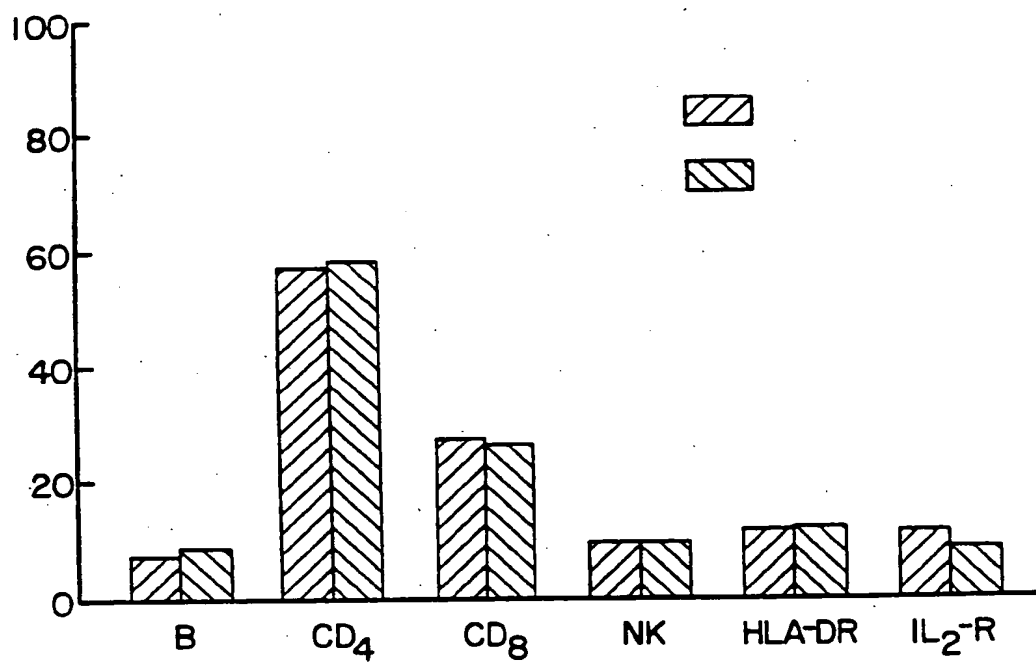


FIG. 3A

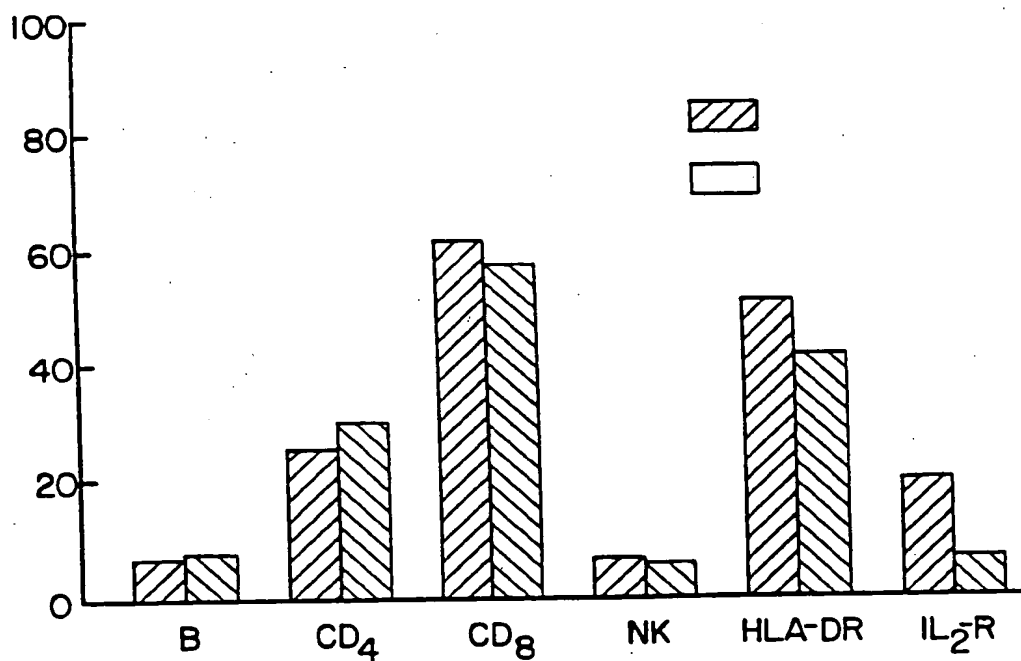


FIG. 3B

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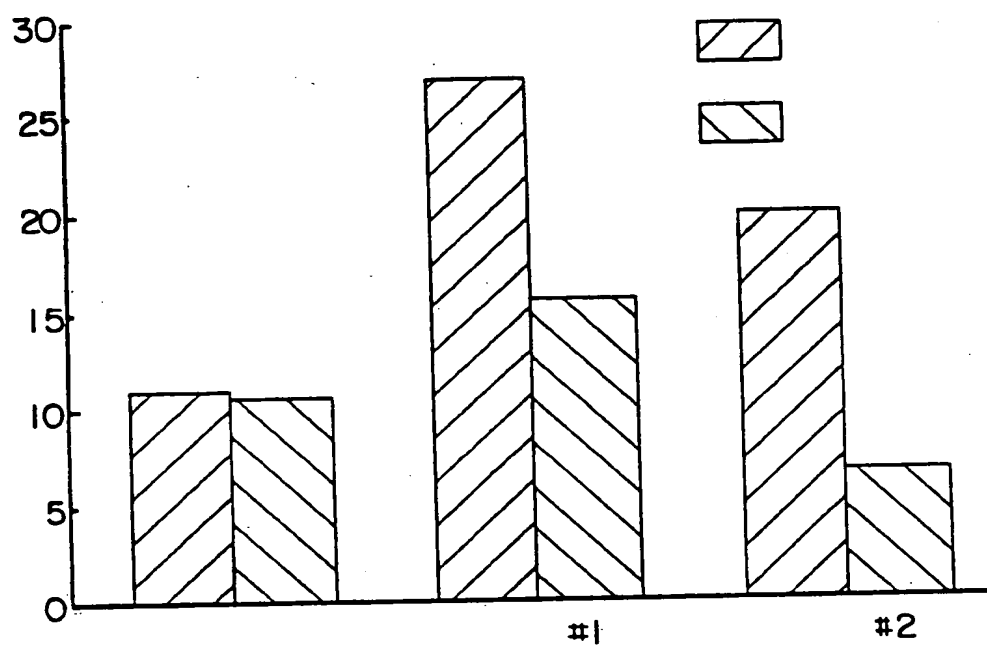


FIG. 3C

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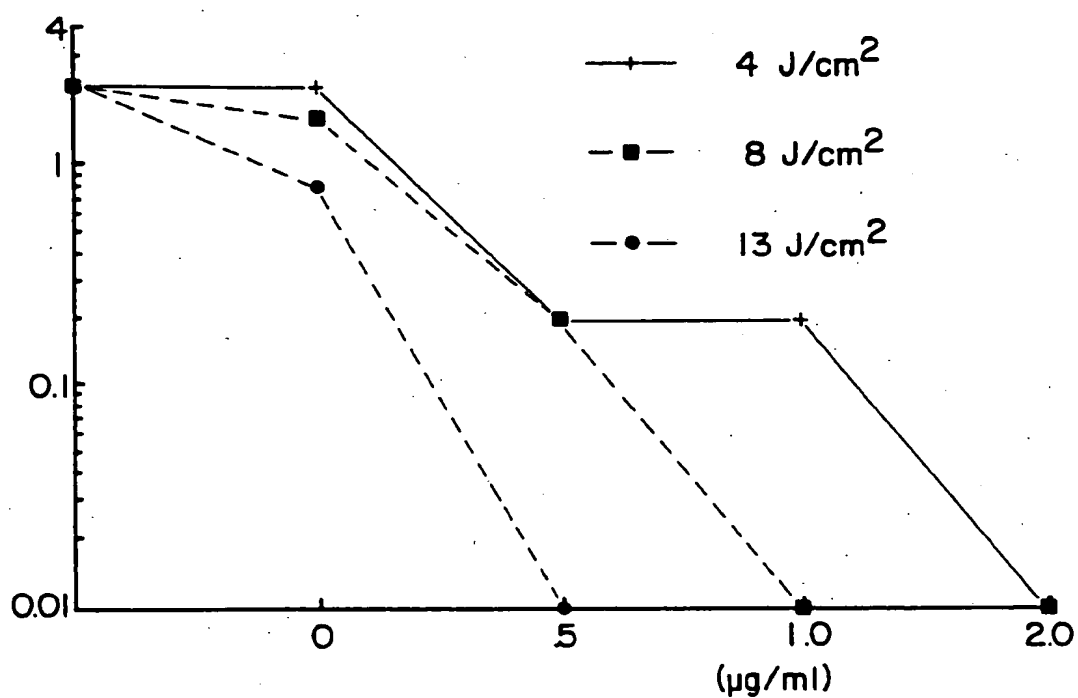


FIG. 4

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FIG. 5-1

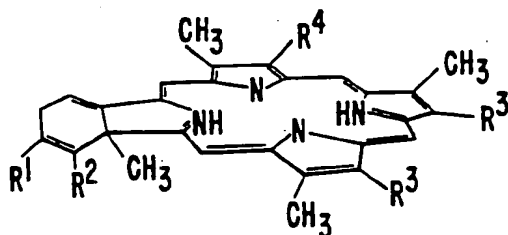


FIG. 5-2

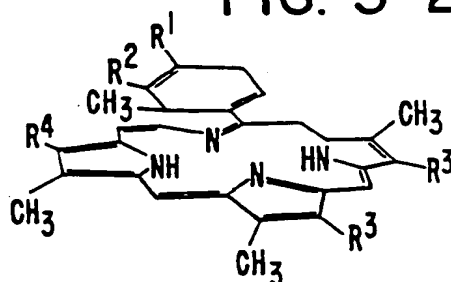


FIG. 5-3

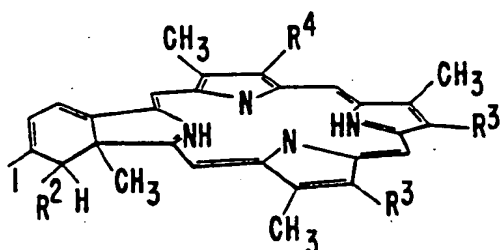


FIG. 5-4

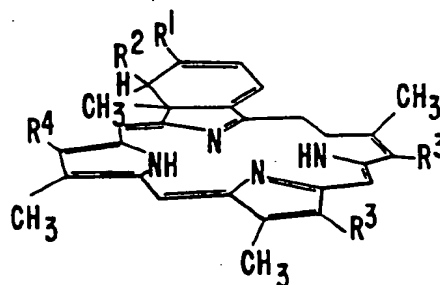


FIG. 5-5

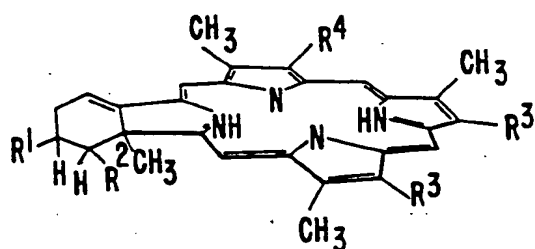
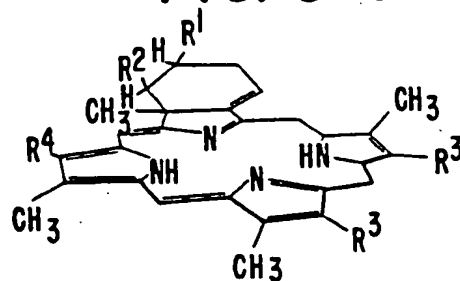
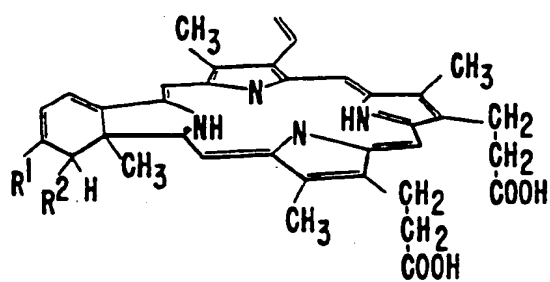
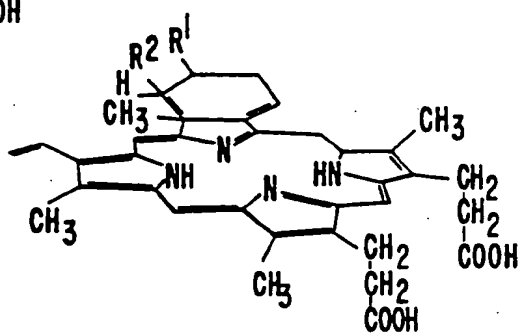
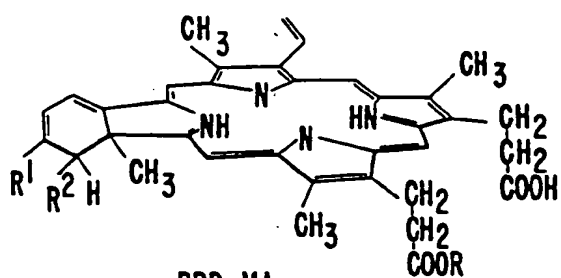
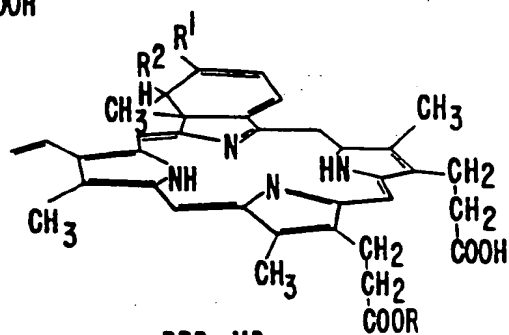


FIG. 5-6



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BPD-DA  
FIG. 6-1BPD-DB  
FIG. 6-2BPD-MA  
FIG. 6-3BPD-MB  
FIG. 6-4

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## INTERNATIONAL SEARCH REPORT

PCT/CA 93/00215

International Application No

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 A61K31/40; A61K41/00		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>9</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	US,A,5 095 030 (LEVY ET AL.) 10 March 1992 cited in the application see the claims, see column 15, line 12 - column 16, line 38	5-8,13
X	* " *	10-12
X	TISSUE ANTIGENS vol. 27, 1986, pages 147 - 154 S. GRUNER ET AL. 'Inhibition of HLA-DR antigen expression and of the allogeneic mixed leukocyte reaction by photochemical treatment' * see the abstract, page 150 Figure 2, page 151 Table 2, discussion*	1-3
Y	* " *	5-8
<p><sup>9</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
17 AUGUST 1993	22. 09. 93	
International Searching Authority	Signature of Authorized officer	
EUROPEAN PATENT OFFICE	B. ISERT	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	<p>TRANSFUSION vol. 32, no. 2, February 1992, pages 121 - 128 J. NORTH ET AL. 'Photodynamic inactivation of retrovirus by benzoporphyrin derivative: a feline leukemia virus model' * see the whole document *</p> <p>---</p>	10-13
X	<p>J. CELL. BIOCHEM. vol. SUP 0, no. 16PE, 3 April 1992, page 85 J. NORTH ET AL. 'Photoinactivation of human immunodeficiency virus (HIV) by benzoporphyrin derivative' * see the abstract*</p> <p>---</p>	13
Y	<p>---</p>	10-12
X	<p>TRANSFUSION vol. 31, 1991, pages 636 - 641 J.L. MATTHEWS ET AL. 'Preliminary studies of photoinactivation of immunodeficiency virus in blood' * see the abstract, Figure 1*</p> <p>---</p>	13
Y	<p>---</p>	10-12

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

CA 9300215  
SA 74513

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-5095030	10-03-92	US-A- 4920143	24-04-90
		JP-A- 2149519	08-06-90
		AU-B- 618725	09-01-92
		AU-A- 1038888	21-07-88
		EP-A- 0276121	27-07-88
		JP-A- 63277700	15-11-88
		US-A- 4883790	28-11-89
		US-A- 5171749	15-12-92
		AU-B- 638675	08-07-93
		AU-A- 3825889	08-02-90
		EP-A- 0352076	24-01-90
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